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MUTATION OCCURRENCE IN PTGS2GENE SEQUENCE IN CERVICITIS DUE TO TOXOPLASMOSIS AND CYTOMEGALOVIRUS INFECTIONS

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Abstract

In this study, venous blood samples were obtained from 100 women with Cytomegalovirus infection whose ages ranged from 18 to 60 years, and 100 healthy women as a control group. The study was conducted at Al-Elwiya teaching hospital in Baghdad city during the period from 1st November 2021 to 15th June 2022. the distrbution of study groups according to age revealed a significant difference (P=0.01) in the cervicitis patient group (34.43±11.89) in comparison with the control group (39.05±13.53). The distrbution of study groups according to the residency showed that residents of rural areas were 51(51.0%) in comparison with the control group and residents of urban areas were 49(49.0%), while the healthy control group was 51(51.0%) with no significant differences (P=0.088). A highly significant differences were bserved between the mean levels of tumor marker Ca125 in patients (25.5±0.71) in comparison with the healthy controls (19.40±0.68), and between mean levels of Ca15.3 (20.80±0.74) and the healthy control group (18.34±0.77). In addition, the mean levels of CRP was (88.06±5.88) versus the controls (6.73±0.12), with a highly significant differences (P<0.001). Also, no significant differences were seen between mean levels of Ca19.9 marker (19.62±0.79) and the control group (19.21±0.71) (P= 0.7). The distribution of polymorphism according to complete blood picture was shown in table (3) .The mean lymphocyte count was 12(60%), while there was a decrease in Hb levels 3(15%), PCV value 4(20%) and Nuetrophil count 2(10%), with no significant differences. there was no mutation occured in the analysis of rs888160762 SNP of PTGS2 gene, but showed a mutation occured in the analysis of rs20417 SNP of PTGS2 gene using Sanger sequencing. The single “C” peak indicates C homozygous alleles, while the presence of “G” and “C” peaks indicates G/C heterozygous alleles.

Keywords: Mutation, PTGS2gene, Sequence, Cervicitis, Toxoplasmosis Cytomegalovirus.

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Introduction

There are pathologic effects of inflammation, due to Toxoplasmosis and Cytomegalovirus infections may lead to neoplastic diseases on the cervix. Cervical non-neoplastic lesions are commonly seen in women of all age groups, but are more commonly observed among sexually active women [1]. These non-neoplastic lesions are either inflammatory lesions or tumor-like lesions (endometriosis, endocervical polyps, Nabothian cysts or endo-cervical hyperplasia). The inflammatory lesions are chronic granulomatous cervicitis or acute and chronic cervicitis that may become infective or non-infective again [2]. Cervical carcinoma often occurs in the fifth decade of life which infected with viral infection [3]. Cervical cancer is a serious global health problem, especially in developing countries such as India [3, 4]. Approximately 90,000 new cervical cancers are being reported in India annually [3]. In India, cervical cancer is diagnosed late, resulting in decreased survival rates. This is because of the lack of screening procedures, misconceptions about gynecological disorders as well as poor awareness on cervical carcinoma [4]. The diagnosis of cervical diseases is affected by other factors such as marital status, age, education, children number, income, contraception use, lifestyles, attitudes, limited knowledge about prevention and screening of cervical cancer, poor family supports, low friendly patient-health service [5]. The above-mentioned factors participate in the variation of the spectrum of cervical disorders in the rural areas in comparison with the urban areas, where rare studies are available. It is considered to be related to wit sexually-transmitted pathogens with rates as high as 30–45% in those populations like cytomegalovirus [6]. While less than half of cervicitis cases are thought to be due to Neisseria gonorrhoeae and Chlamydia infections, the causes of the remainder which are known as non-gonococal, non-chlamydial cervicitis or non-specific cervicitis are still unknown [7]. There are also variable pathogenic contributions of *Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Herpes simplex, Cytomegalovirus, Adenovirus* as well as *Trichomonas vaginalis* and Toxoplasmosis [8]. Considering this issue and limited information on the possible impacts of TTMV on both cervicitis and cervical carcinomas, this study aimed to determine the frequency of TTMV in among cases of cervicitis and cervical carcinomas and their possible association with them [12]. This study aimed to detect mutation occurrence in PTGS2 gene.

Materials and Methods

In this study, venous blood samples were obtained from 100 women with Cytomegalovirus infection whose ages ranged from 18 to 60 years, and 100 healthy women as a control group. The study was conducted at Al-Elwiya teaching hospital in Baghdad city during the period from November 2021 to June 2022.

The blood samples were divided into two parts, the first part was put in EDTA tubes for hematological investigations (CBC), and PCR technique, the second part was placed in plane tubes, then centrifuged after clotting at 3000 rpm for 15 minutes to obtain serum, which is stored at -20C until use.

Toxoplasma gondii and Cytomegalovirus infection were detected by screening test.

Detection of CA 19-9, CA- 125 II and CA 15-3 II markers was performed using CA 19-9 CalSet, Elecsys CA- 125 II and Elecsys CA 15-3 II kits and Cobas E411 analyzer, while the PCR test was detected using the CRP kit and Avidas CRP instrument.

The conventional PCR was used for gene detection and DNA sequencing of the patient's samples.

For Gene detection and DNA sequencing by PCR the Primers used:

Primer Name	Sequence 5`- 3`	Annealing Temp. (°C)	Product Size (bp)
COX2-F	TGTAAAACGACGGCCAGTCTGAGCACTACCCATGATAGA	55	760
COX2-R	CAGGAAACAGCTATGACGGGCGAGTAAGGTTAAGAAAG		

Statistical analysis: The SPSS program was used to perform the statistical analysis. Data were provided as mean±SD or numbers and percentages. The Student t-test was used for comparison between abortion cases and normal individuals.

Results

Table (1) showed that the distrbution of study groups according to age revealed a significant difference (P=0.01) in the cervicitis patient group (34.43±11.89) in comparison with the control group (39.05±13.53). The age group (15-24) years was 24 (64.9%) compared to the control group 13(35.1%), while the age group (25-34) years formed 34(49.3%) compared to the control group 35(50.7%), and the age group (35-44) years was 18(58.1%) compared to the control group 14(41.9%). The results also showed that the age group (55-60) years was 6(25%) in comparison with the control group 18(75%).The distrbution of study groups according to the residency showed that residents of rural areas were 51(51.0%) in comparison with the control group and residents of urban areas were 49(49.0%), while the healthy control group was 51(51.0%) with no significant difference (P=0.088).

Table (1): Demographical picture of study groups (N=200)

Parameters		Case (N=100)	Control (N=100)	P-value
Age (M±SD)		34.43±11.89	39.05±13.53	**0.01 (S)**
Age (Years)	(15-24)	24 (64.9%)	13 (35.1%)	**0.03 (S)**
	(25-34)	34 (49.3%)	35 (50.7%)	
	(35-44)	18 (58.1%)	14 (41.9%)	
	(45-54)	18 (47.4%)	20 (52.6%)	
	(55-60)	6 (25 %)	18 (75 %)	
Residency	Rural	51 (51.0%)	49 (49.0%)	**0.088(N.S)**
	Urban	49 (49.0%)	51 (51.0%)	

S : significant N.S: no significant

A highly significant difference was found between mean levels of tumor marker Ca125 in patients (25.5±0.71) in comparison with the healthy controls (19.40±0.68), and between mean levels of Ca15.3 (20.80±0.74) and the healthy control group (18.34±0.77). In addition, the mean levels of CRP was (88.06±5.88) versus the controls (6.73±0.12), with a highly significant differences (P<0.001). Also, no significant difference was found between mean levels of Ca19.9 marker (19.62±0.79) and the control group (19.21±0.71) (P= 0.7) as shown in table (2).

Table (2): The mean differences in the level Of tumor markers between the study groups

Tumor marker	Study Groups	N	Mean	Std. Error Mean	T-test	P-value
CA19.9 (37U/mL)	Case	100	19.62	0.79	0.38	0.7 (N.S)
	Control	100	19.21	0.71		
CA125 (35U/mL)	Case	100	25.25	0.71	8.4	<0.001 (H.S)
	Control	100	19.40	0.68		
CA15.3 (35U/mL)	Case	100	20.80	0.74	3.3	0.02 (S)
	Control	100	18.34	0.77		
CRP (10mg/L)	Case	100	88.06	0.77	13.0	<0.001 (H.S)
	Contro l	100	6.73	0.12		

S : significant N.S: no significant H.S: highly significant

The distribution of polymorphism according to complete blood picture was shown in table (3) .The mean lymphocyte count was 12(60%), while there was a decrease in Hb levels 3(15%), PCV value 4(20%) and Nuetrophil count 2(10%), with no significant differences. as shows in table (3).

Table (3): Complte blood picture profile and occurence of polymorphism among patient group (N=100)

Polymorph-ism	Lymphocyte		Hb		PCV		Neutrophil		
	Normal count	High count	Normal levels	High levels	Normal levels	High levels	Normal count	High count	Low count
Yes (N=20)	8 (40%)	12 (60%)	17 (85%)	3 (15%)	16 (80%)	4 (20%)	3 (15%)	2 (10%)	15 (75%)
No (N=80)	32 (40%)	48 (60%)	16 (20%)	64 (80%)	34 (42.5%)	64 (57.5%)	29 (36.3%)	15 (18.7%)	36 (45%)
P-value	1.0		0.6		0.3		0.04		

S : significant N.S: no significant

Amplification of Cox2 regen in women with cervicitis

By using PCR technicuqe, the amplification of Cox2 region in women with cercivitis was factionated on 1.5% agarose gel electrophoresis, stained with Ethedium bromide M:100bp ladder marker Lanes 1-10 and Lanes 11-20 resembling 760bp PCR-product, as illustrated in figure (1).

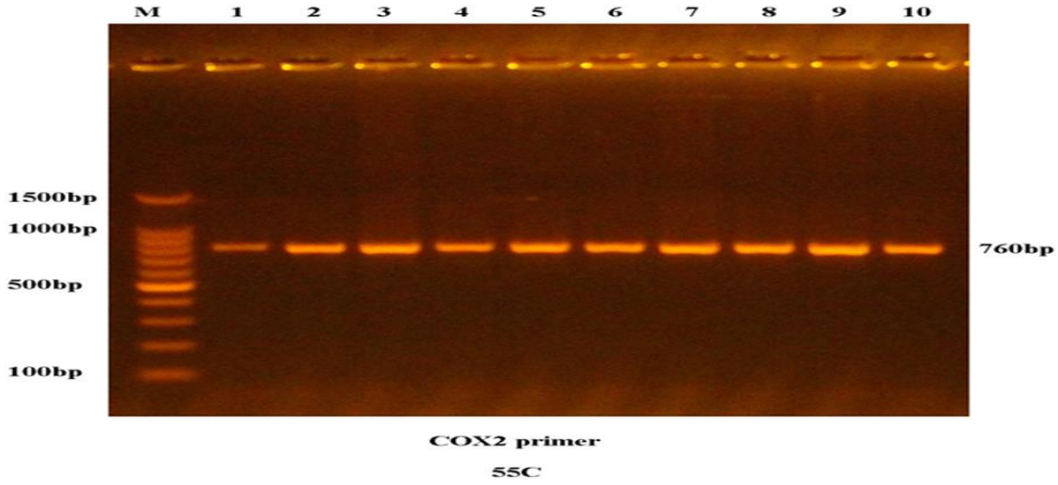

Figure (1): The amplification of Cox2 region in women with cercivitis were factionated on 1.5% agarose gel electrophoresis, stained with Ethedium bromide M:100bp ladder marker Lanes 1-10 resembling 760bp PCR-prodect.

Table (4) and figure (2) revealed that there was no mutation occured in the analysis of rs888160762 SNP of PTGS2 gene, but showed a mutation occured in the analysis of rs20417 SNP of PTGS2 gene using Sanger sequencing. The single "C" peak indicates C homozygous alleles, while the presence of "G" and "C" peaks indicates G/C heterozygous alleles.

Table (4) showed the mutation occurrence in PTGS2 GENE ID 5743 SNPs rs20417, Wild CC GG and the Variation C>G G>C compared to healthy control.

PTGS2 GENE ID 5743				
SNPs	patients rs888160762	patients rs20417	Control rs888160762	Control rs20417
Wild	CC	GG	CC	GG
Variation	C>G	G>C	C>G	G>C
Samples				
1	CC	GG	CC	GG
2	CC	GC	CC	GG
3	CC	GC	CC	GG
4	CG	GC	CC	GG
5	CC	GC	CC	GG
6	CC	GG	CC	GC
7	CC	GC	CC	GG

8	CC	GC	CC	GG
9	CC	GG	CC	GG
10	CC	GC	CC	GC
11	CC	GC	CC	GG
12	CC	GG	CC	GG
13	CC	GC	CC	GG
14	CC	GC	CC	GG
15	CC	GC	CC	GG
16	CC	GG	CC	GG
17	CC	GG	CC	GG
18	CC	GC	CC	GG
19	CC	GC	CC	GG
20	CC	GC	CC	GC

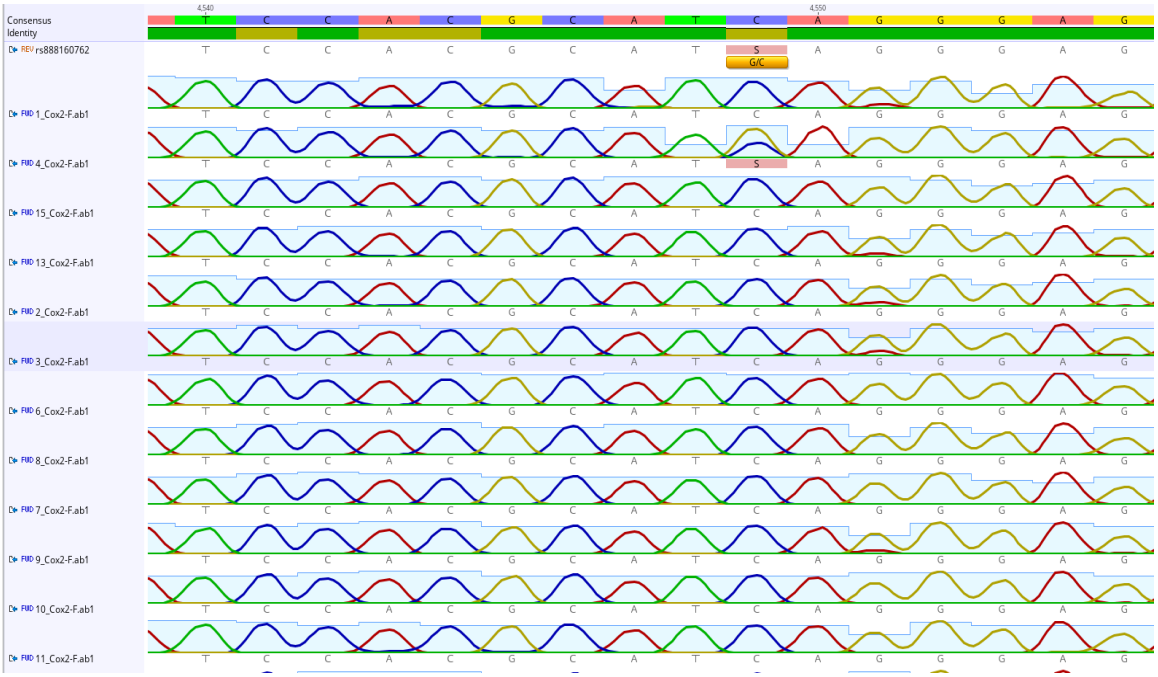

Figure (4-5): Analysis of rs888160762 SNP of PTGS2 gene using Sanger sequencing. The single "C" peak indicates C homozygous alleles, while the presence of "G" and "C" peaks indicates G/C heterozygous alleles

Discussion

According to the results of the current study, the distrbution of the patient groups according to the age groups was 34.43±11.89 in comparison with the control group, (39.05±13.53). The ages (25-34), 34 (49.3%), were more prevalent in women with cecivitis than other ages. Thes findings disagreed with (Patricia *et al.*, 2016), [10]; who reported that the prevalence of study group according to ages were (57.1%), and (Zhang, *et al.*, 2018), [11]; who reported that among women with cervical intraepithelial lesions aged (65–69) years was (38.8%) which was significantly higher than that of the other age groups**.** The mean tumor marker Ca125 was shown to be highly significant among patients with a mean age of (25.25±0.71) in comparison with the healthy controls (19.40±0.68), and these results matched with (Charkhchi, *et al.,* 2020) [11], who reported that the tumor marker Ca125 has been used as the primary ovarian cancer marker for the past four decades and gave a highly significant levels. Also the mean of Ca15.3 (20.80±0.74) and the healthy control group (18.34±0.77), but no significant differences were found between mean levels of Ca19.9 marker (19.62±0.79) and the control group (19.21±0.71). While the mean levels of CRP was (88.06±5.88) versus the controls (6.73±0.12), with no significant difference. These findings agreed with (Orsolini, *et al.*, 2022) [12], who explained that the CRP levels were highly incresed in cervicitis and overian cancer, on the other hand, there is a high level in the measurements of Ca125 marker with these serious cases. Bian, *et al.*, (2017) [13]; stated that the Ca125 levels were high in patients with endometrial cancer or cervicitis that develops into cancer. According to the results of complete blood count, the lymphocyte count increased at (60%), which corresponds to the occurrence of inflammation. This is clear evidence that there is a defect in the human body represented by a genetic mutation and that the increase in lymphocytes may evident of cancer in the body, which amounts to neoplasia. Milne, et al., (2012) [14]; indicated that there is an increase in the number of lymphocytes in women suffering from ovarian cancer, and this indicates the occurrence of genetic mutations in those organs of the body that may reach the Neoplasia [15]. The mutation occurence for analysis of rs20417 NSP of PTGS2 gene with wild CC GG and the variation C>G G>C. The genetic variations of SNPs in PTGS2 genes was found to have ability to create tissue milieu favoring tumor digenesis. Other studies indicated that the common SNP rs20417 is associated with PCa risks. The results of Zhang, et al., (2015). [16]; found association between SNP rs20417 and PCa risks either at genotypical levels or at allele levels (CC > GG, 1.00, 95% CI 0.87 to 1.15; CC + GC > GG, 95% CI 0.96 to 1.06; CC > GC + GG, 95% C>G, 95% GC> GG, 95% (Zhang, et al., 2015); [17].

Conclusions

The polymorphism occurence in analysis of rs20417 SNP of PTGS2 gene with wild CC GG and the variation C>G G>C. The genetic variations of SNPs in PTGS2 genes was found to have ability to create tissue milieu favoring tumor digenesis.

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